3/10/2015

Tulane National Primate Research Center (TNPRC) Incident

Wildlife sampling protocol

Recommendations Short-Term and Long-Term Monitoring

This wildlife sampling protocol is a multi-agency effort including various Federal and State wildlife and agriculture agencies. Wildlife can transport *Burkholderia pseudomallei* into new environments. Wildlife may also serve as sentinels for the presence of *B. pseudomallei* in the environment, indicating the presence of the organism in the environment and risk to humans or other animals. This recommendation provides a mechanism to monitor the risk of *B. pseudomallei* to humans and other animal. This wildlife sampling should be done in concert with soil sampling to assist with interpretation and further quantify the likelihood of *B. pseudomallei* outside the Tulane National Primate Research Facility.

Actions in this plan will be carried out in a coordinated fashion. Oversight will be the primary responsibility of Louisiana Department of Wildlife and Fisheries, with input from the working group as needed. The United States Department of Agriculture National Veterinary Services Laboratories will perform the testing of samples in collaboration with other laboratories as needed. This plan will be carried out as recommended for a period of 5 years or until acceptable level of confidence is reached, under the assumption that surveillance results in all negative culture results, presumptive positives do not exceed the expected threshold, and soil sampling around presumptive positives do not indicate the presence of the *B. pseudomallei* in the environment.

Short-Term Objectives*:*

- Establish the baseline for the extent of *B. pseudomallei* and other cross reacting organisms outside of the TNPRC in wildlife.
- Determine whether rodents and feral cats have been exposed or are infected.
- Determine whether wildlife are able to transmit the organism.
- Establish the baseline for sentinel surveillance sites.
- Develop a monitoring plan using wildlife as sentinel proxies.

Complete short-term objectives by the end of April 2015 with the number of samples required.

Short-Term Actions*:*

1. Collect wildlife samples (and samples from TNPRC feral cats) within the perimeter fence of TNPRC
    a. 100 rat/mice, 10 nutria, 60 birds, 20 other wildlife, all TNPRC cats
    b. Divide wildlife animal collections equally among North and South Campus
2. Collect wildlife samples on Tulane property outside of the perimeter fence of TNPRC
    a. 30 rat/mice, 30 other wildlife including nutria

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   b. Locations approved by LDWF

3. Collect wildlife samples from three control sites
   a. 30 rat/mice, 30 other wildlife including nutria
   b. Sites include the Fairgrounds, Bogue Falaya Park, and Fairview Riverside State Park
4. Monitor wildlife population within a 2-mile perimeter and at control sites
   a. Submit any dead or sick wildlife found to USGS National Wildlife Health Center for examination and diagnosis, if *B. pseudomallei* is suspected, send samples to USDA National Veterinary Services Laboratories
5. Develop sample sizes for long-term monitoring

Long-Term Objectives

- Monitor for the presence of *B. pseudomallei* in the environment via wildlife as sentinels.
- Monitor the feral cat and rodent population on the TNPRC.
- Obtain tissues and serum for understanding of tests in wildlife (cross reactions, etc.).

Complete long-term objectives over 5 years, or until an acceptable level of assurance (as set by the working group) that B. pseudomallei is not present in the environment is reached.

Long-Term Actions

- Continue to sample wildlife in TNPRC, outside the perimeter fence but on the property, and in the three control locations at defined intervals.
- Continue to monitor for moribund or found dead wildlife.
- Conduct analyses of test results to develop better methods for interpreting serology and culture results.

Tulane Incident Domestic Animal Surveillance Working Group

USDA:
Katie Portacci, VS
Tim Clouse, VS
Lily Rai, VS
Mike Logan, VS
Matt Erdman, VS
Ken Angel, VS
Freeda Isaac, VS
Kathe Bjork, VS
Alejandro Rooney, ARS
Carol Clarke, AC

DOI:
Leann White, USGS
Bob Dusesk, USGS

St. Tammany Parish:
Dexter Accardo
HHS:
Jay Gee, CDC
Miguel Cruz, CDC
Danielle Mills, CDC

LA:
Jeff Dauzat, LDEQ
Jim LaCour, LDWF
Diane Stacy, LDAF
John Roberts, LDAF
Brent Robbins, LDAF

Tulane:
Skip Bohm
Don Sibley
Shelley Falkenstein

Supporting Documents:

Appendix A Implementation

Appendix B NVSL sample submission protocol

Appendix C USGS National Wildlife Health Center Sample Submission protocol

Appendix D *Burkholderia pseudomallei* suspect identification guide

Appendix E Wildlife case interpretation

Appendix F Suggested trap locations for wildlife on Tulane property

Appendix A. Implementation

Sample collection:

Wildlife will be collected in live capture traps by Tulane (or Nuisance Wildlife Control Operator (NUCO)) as a part of active surveillance. Sites will be approved by Louisiana Department of Wildlife and Fisheries (LADWF) (Appendix F). Animals will be taken in their traps to TNPRC, and animals will be humanely euthanized. One truck will be designated for picking up the traps each day on the Tulane property. Traps will be washed in the cage wash prior to leaving the facility and returning to the trap site. Tulane pathologists will collect and submit tissues according to the USDA:APHIS:VS: National Veterinary Services Laboratories protocol Appendix B.

A database will be developed to track each animal with an individual animal ID assigned to each animal. The database will also capture the GPS coordinates of the trap site, antemortem disposition (healthy or ill), and postmortem description (no lesions, suspect lesions, unrelated lesions).

A passive component will also compliment the active surveillance in all sites. All found dead or moribund wildlife will be submitted directly to USGS National Wildlife Health Center (NWHC) for necropsy. Tulane (or LADWF) will submit samples per USGS protocol Appendix C. USGS will identify cause of illness or death in wildlife. GPS coordinates should be collected and animals entered into the Tulane database for active surveillance. In any animals without a clear diagnosis, USGS will do the initial culture according to the recommended methods in Appendix D and submit suspect cultures to NVSL for speciation.

Short term samples:

All samples to be collected by 4-30-15. Tulane is encouraged to continue submitting wildlife samples once quota is reached.

Within the Tulane Perimeter fence

- 100 rat/mice (priority sample)
  - stratified 50:50 North: South campus
  - 10 small rodent traps have been provided by USGS until Tulane can purchase traps
- 20 other wildlife species
  - divided by N and S campus (opossum, skunks, raccoon, coyote)
  - Permits have already been obtained for contractors
- 10 nutria from Tulane Wetland (site TBD) *
- 60 birds
  - Cage will be placed close to infected primate cages on south campus
  - Cage sent by NWHC
- Census of cats
  - All cats will be captured, sedated for serum and urine collection.
  - Cats (especially hard to catch cats) will be held while culture results are pending
  - *Per USDA:APHIS:Animal Care, IACUC approval is not required for regulatory testing of these cats*

Tulane property outside the perimeter fence:

- 30 aggregate rat/mice among
- 30 aggregate wildlife including nutria

Control sites

- 30 aggregate rat/mice divided between sites
- 30 aggregate wildlife including nutria
- Fairgrounds, Bogue Falaya Park, and Fairview Riverside State Park
- *Tulane will put trap sites near Northlake Christian School to monitor wildlife in that direction.*
- Local authorities will get permission for trapping, LADWF will facilitate permits, Tulane will contract out the collection.

Wildlife population at large

- Monitor any die-offs within the watershed, particularly after periods of heavy rain and wind
- LADWF will monitor morbidity and mortality events in wildlife within a 2 mile radius of the TNPRC, Tulane will monitor on Tulane-owned property

Long term samples:

Monitoring for a period of 5 years or until acceptable level of assurance (as determined by the working group) is reached.

Sampling plan is currently being developed for long term.

Sampling will continue as planned as long as no confirmed cases are identified, soil samples do not identify the organism, and presumptive positives do not exceed an expected threshold.

The Tulane cats will be monitored along with wildlife.

July 2015
October 2015
May 2016
October 2016
May 2017
October 2017
May 2018
October 2018
May 2019
October 2019

Diagnostics:

Urine and tissue will be submitted to NVSL per the protocol outlined. Tissues and urine will be cultured. Speciation of any growth will be conducted on any cultures. Serology/IHA will be conducted in accordance with the protocol identified by NVSL and Australia on all active surveillance specimens. The current capacity for NVSL is 15 animals (3 samples per animal) or 50 samples per week.

Interpretation:

Interpretation of wildlife will follow the case definition Appendix E. The working group recommends all presumptive positives and confirmed cases will be followed with soil sampling near the trap site (need direction for experts on what the protocol should be). Due to the characteristics of the test, interpretation is not as clear negative or positive. Other organisms may cross react with serology, leading to false positives. The organism can also hide in other cells leading to false negative results. These characteristics will be accounted for in the estimation of a sample size to

achieve detection. In addition, an expected amount of false positives will be reported with the long term sampling plan. This will serve as a threshold of acceptable false presumptive positives (positive on serologic tests but negative on culture) and exceeding this threshold will serve as an indicator to reevaluate this surveillance plan.

All Tulane managed cats will be release if culture results are negative (classified as presumptive negative). The working group will need to discuss interpretation of presumptive positives in greater detail if that occurs.

In all wildlife where culture is negative and serology is pending, these samples will be classified as presumptive negative until serologic results are back. Culture positives will always result in a confirmed case independent of serology.

Monitoring:

LADWF will be responsible for monitoring the long-term progress of sampling.

Tulane will maintain a database of all submissions, identifications, locations, and results. This will be shared with Federal/State partners as needed.

Negative results will be discussed at the next scheduled working group call.

Appendix B. NVSL sample submission protocol

Sample collection and submission to NVSL for Burkholderia pseudomallei isolation and serology

Collect no more than 50 individual samples for culture and 50 for serology (blood in red top tubes) daily, and no more than 100 samples for culture per week. These numbers may be adjusted over time. Samples can be submitted starting 2/24 (for arrival 2/25). Testing takes 5-10 days.

Sample collection:

- All samples must have a primary (tube or bag) and secondary (bag) layer of containment with absorbent material. Label all tubes and bags with animal ID and sample type.
- Tissue:
    - Collect 1-10 grams (pea sized to walnut size) of each tissue, depending on the size of the animal.
    - Tissues should be collected individually in whirl-pak bags/ziplock bags or other securely closing container such as 50ml screw cap plastic tube. Close bags tightly.
    - Disinfect outside of bags or tubes. Bag sets of tissues by animal along with absorbent material (paper towels work well) in a second securely closing plastic bag (ziplock or whirl-pak).
- Blood and urine:
    - Serum does not need to be separated from the clot but tube should be centrifuged if possible.
    - Rubber stopper-type tubes must have the stopper secured with parafilm or tape (electrical or medical). Screw top tubes must be closed tightly.
    - Red top tubes and green top tubes may be shipped together.
    - Blood tubes should be placed in a secure transport container such as a 40 slot fiberboard box so that individual tubes do not make contact.
    - Place box into a securely closing plastic bag with absorbent material.

Samples to collect:

Smaller animals – sacrificed:

- Blood (if possible) – red top tube for serology – as much as can be obtained
- Collect visible abscesses/lesions in any tissue including skin
- If no lesions are present, collect:
    - Liver and lung (pool)
    - Urine (Bladder if urine cannot be collected)

Larger animals – non-sacrificed:

- Aspirate or biopsy from any visible abscesses – red top tube
- Blood for serology – red top tube – 1-2ml minimum
- Whole Blood for culture – green top (heparinized) tube – at least 1ml
- Urine for culture – (in order of preference)by cystocentesis, catheterization, or expressed – at least 1-2ml in a red top tube

How to ship:

- Ship samples on day of collection overnight on ice packs. Separate samples in bags from ice packs slightly with packing peanuts or crinkled paper towels to prevent freezing. Ship for arrival Tuesday-Friday. **No** Saturday Arrival!
- Ship in a Category B (UN3373) compliant box at a minimum. NVSL can provide shipping containers with prepaid labels if needed – contact the Bacterial Identification lab at 515-337-7565.
- Fill out the attached APHIS 10-4 submission form – some fields have been pre-filled. The listed submitter will receive all reports. Boxes 1, 4, 10, 11, 12, 15 (FAD #), and 17-21 should be filled out. Include a copy in the package so that it is easily accessible without opening sample bags.
- Ship to: UNITED STATES DEPARTMENT OF AGRICULTURE
  ANIMAL AND PLANT HEALTH INSPECTION SERVICE

  NATIONAL VETERINARY SERVICES LABORATORIES

  P.O. BOX 844, 1920 DAYTON AVENUE, AMES, IA 50010

  (515) 337-7514

- Send a copy of the 10-4 and tracking number by e-mail to the following on the day they are shipped:
  Linda.k.schlater@aphis.usda.gov; ginger.r.harvey@aphis.usda.gov; Kristina.lantz@aphis.usda.gov; NCAH.samples@aphis.usda.gov. If it is necessary to send by fax, call first to 515-337-7565 to notify of the fax, and then send the fax to 515-337-6528.

Appendix C. USGS National Wildlife Health Center Sample Submission protocol

OIE Collaborating Centre for Research,
Diagnosis and Surveillance of Wildlife Pathogens

USGS-National Wildlife Health Center

Diagnostic Submission Guidance and Assistance
for Sampling around Tulane Primate Research Center

- In addition to specimens that meet our normal submission guidelines (http://www.nwhc.usgs.gov/services/USGS_NWHC_Diagnostic_Case_Submission_Guidelines.pdf), the USGS-National Wildlife Health Center (NWHC) will accept any single dead or moribund free-ranging (native, introduced, feral) species without an obvious cause of death within a 2 mile radius of the Tulane National Primate Research Center for cause of death determination.
    - Diagnostic tests associated with cause of death determination will be performed by the National Wildlife Health Center. If preliminary test results indicate the potential presence of a select agent, samples will be sent to the USDA National Veterinary Services Laboratory (NVSL) for confirmatory testing. If presence of a select agent in the sample is declared by NVSL all remaining samples from the carcass will be secured in BSL-3 space at NWHC and destroyed, inactivated, or transferred as per select agent requirements.
- Collection and shipment of carcasses should follow procedures outlined in NWHC's Shipping guidelines. Briefly:
    - Contact NWHC to notify of shipment (nwhc-epi@usgs.gov; 608-270-2480)
    - Label (date, location, field signs, etc.) individual carcasses and double bag.
    - Ensure that bags are sealed to prevent leakage of any potentially infectious material.
    - A third bag, containing the double-bagged carcass and blue ice packs, should be used to line the hard sided cooler and then sealed shut.
    - A completed NWHC specimen history form in a plastic baggie should be taped to the top of the container.
    - The outside of the shipping container should be labeled as UN3373, Biological Substance Category, B (carcasses of animals that died of unknown causes)
    - Detailed instructions can be found at http://www.nwhc.usgs.gov/mortality_events/shipping_instructions.pdf
- PPE during carcass collection
    - Disposable gloves, respirators, and boots and clothing that can be removed and disinfected or disposed of (e.g., boot cover, Tyvek) prior to leaving the site are advised
    - Please follow any additional guidelines from your agency for dealing with potential zoonotic pathogens
- Disinfection:

    - Disinfection of gear and equipment (outside of garbage bags, traps, vehicles) is needed to prevent movement of potential pathogens outside of the site
    - Virkon is an effective disinfectant
    - Items should be free of organic debris before disinfecting
    - Disinfection should be done prior to leaving the site
- Please contact the NWHC Field Epidemiology Team (nwhc-epi@usgs.gov, 608-270-2491) if assistance with any of the following is needed:
    - Additional details for carcass collection or disinfection to minimizing human exposure and movement of pathogens
    - Sampling design for detection of exposure and current infections in wildlife (i.e., surveillance of wildlife)
    - Field assistance with collection of surveillance or mortality samples

Appendix D. *Burkholderia pseudomallei* suspect identification guide

B. pseudomallei can be isolated from a wide variety of samples from most animal, reptile, or avian species and environmental sources. Process specimens and handle suspect cultures in a class 2 biosafety cabinet in Biosafety level 2 at minimum. Suspect cultures should be referred to the National Veterinary Services Laboratories for confirmation.

Colony characteristics:

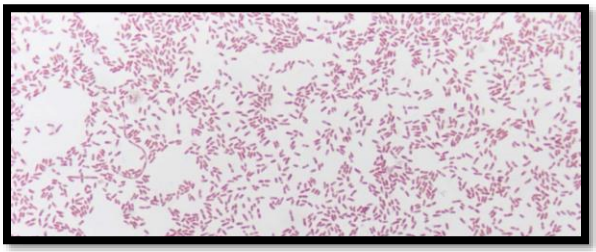
Sheep Blood Agar (SBA): Non-hemolytic, small, smooth creamy white colonies in the first 1 to 2 days, become dry and wrinkled after a few days, similar to Pseudomonas stutzeri. No violet pigment on blood agar

MacConkey: Not always present. If present, small, smooth non-lactose fermenting colonies, pinpoint at 48 hours, become dry and wrinkled in later days

Note: B. pseudomallei usually has a distinct earthy/musty odor which is often noticeable on opening the incubator – individual plates should never be smelled!

Left: Young B. pseudomallei cultures on SBA. Right: Older dry wrinkled colonies on selective media – colonies on blood agar do not produce violet pigment.

Microscopic characteristics:



Small, straight, slightly curved gram negative rods. Occasional bipolar staining in direct samples.

Biochemical characteristics:

Catalase (Perform if not growing on MacConkey): Positive
Oxidase: Positive
Indole: Negative
Motility: Positive
Colistin or Polymyxin B: Resistant, no zone (Most Pseudomonas are susceptible)
Penicillin: Resistant
Arginine dihydrolase: Positive
Nitrate reductase: Positive with gas production

Rapid/Automated/Genomic identification:

Many commercial systems may misidentify or fail to identify B. pseudomallei and should not be considered reliable. Isolates without violet pigment identifying as Chromobacterium violaceum on API 20E or 20NE should be considered highly suspicious for B. pseudomallei.

16S rDNA sequencing cannot reliably differentiate B. pseudomallei, B. thailandensis, and B. oklahomensis

MALDI-TOF Biotyper: Should not be considered reliable as bacterial databases do not contain entries for B. pseudomallei. Isolates may either identify as a poor match to B. cepacia or not identify.

For additional screening information, see the American Society for Microbiology Laboratory Response Network sentinel laboratory culture guidelines at http://www.asm.org/images/PSAB/Burkholderia101714.pdf.

Appendix E. Wildlife case interpretation

Screening:

Serology (+) = **presumptive positive**

Serology (-) = **presumptive negative**

*Confirmatory:**

Sample will always be cultured, regardless.

Serology (+), Culture (+) = **confirmed case-** need soil and wildlife sampling to understand extent

Serology (-), Culture (+) = **confirmed case**- need soil and wildlife sampling to understand extent

Serology (+), Culture (-) = **presumptive positive**- need soil sampling to interpret

Serology (-), Culture (-) = **Negative**

in the situation where culture is negative and serology is pending, these samples will be classified as **presumptive negative until serology results are back.*

Wildlife morbidity/mortality monitoring: *(*NOTE assumption is only wildlife with NO other cause of death will go to NVSL)*

Screening:

Gross pathology indicative of Bpm or no cause of death identified (+) = **Suspect**

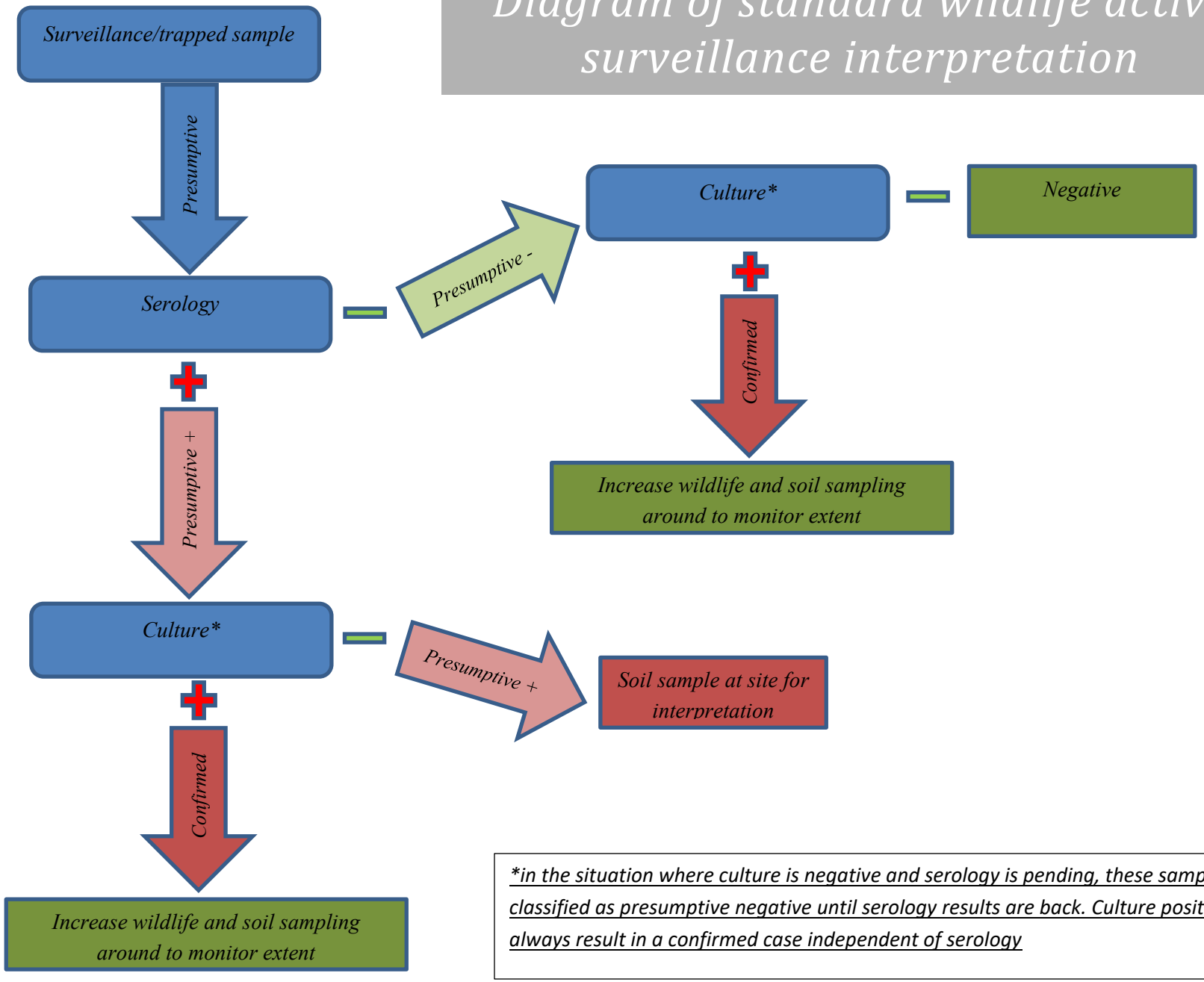
Gross pathology or other diagnostics find another cause of death (-) = **presumptive negative**

Confirmatory:

Suspect, Culture (+) = **Confirmed case**

Suspect, Culture (-) = **Negative** (*Note discussion here that we could have another category of compatible gross pathology suspects get PCR run too?)

Diagram of standard wildlife active surveillance interpretation



*in the situation where culture is negative and serology is pending, these samples will be classified as presumptive negative until serology results are back. Culture positives will always result in a confirmed case independent of serology

Appendix F. Suggested trap locations for wildlife on Tulane property

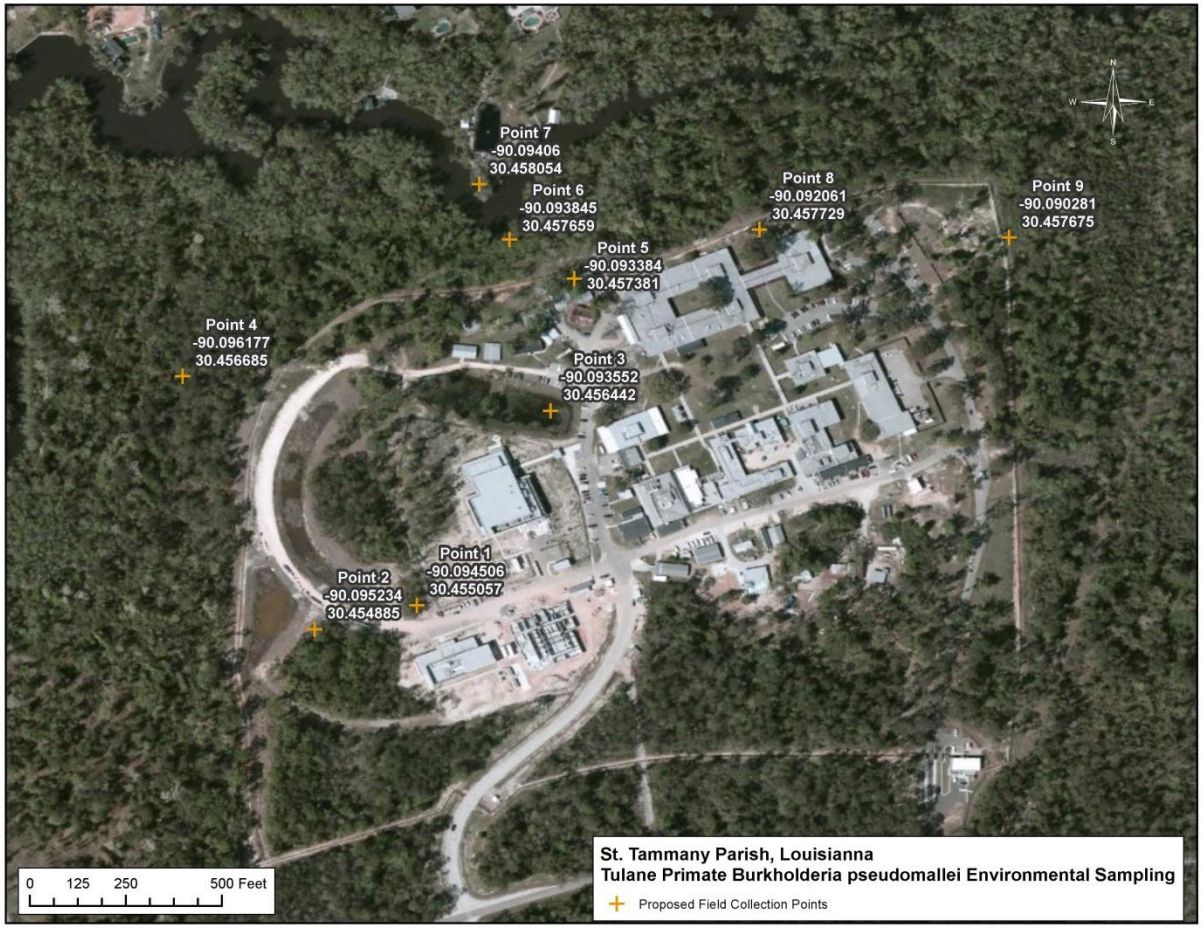


Figure 1 suggested locations of placement of wildlife traps on the TNPRC North Campus

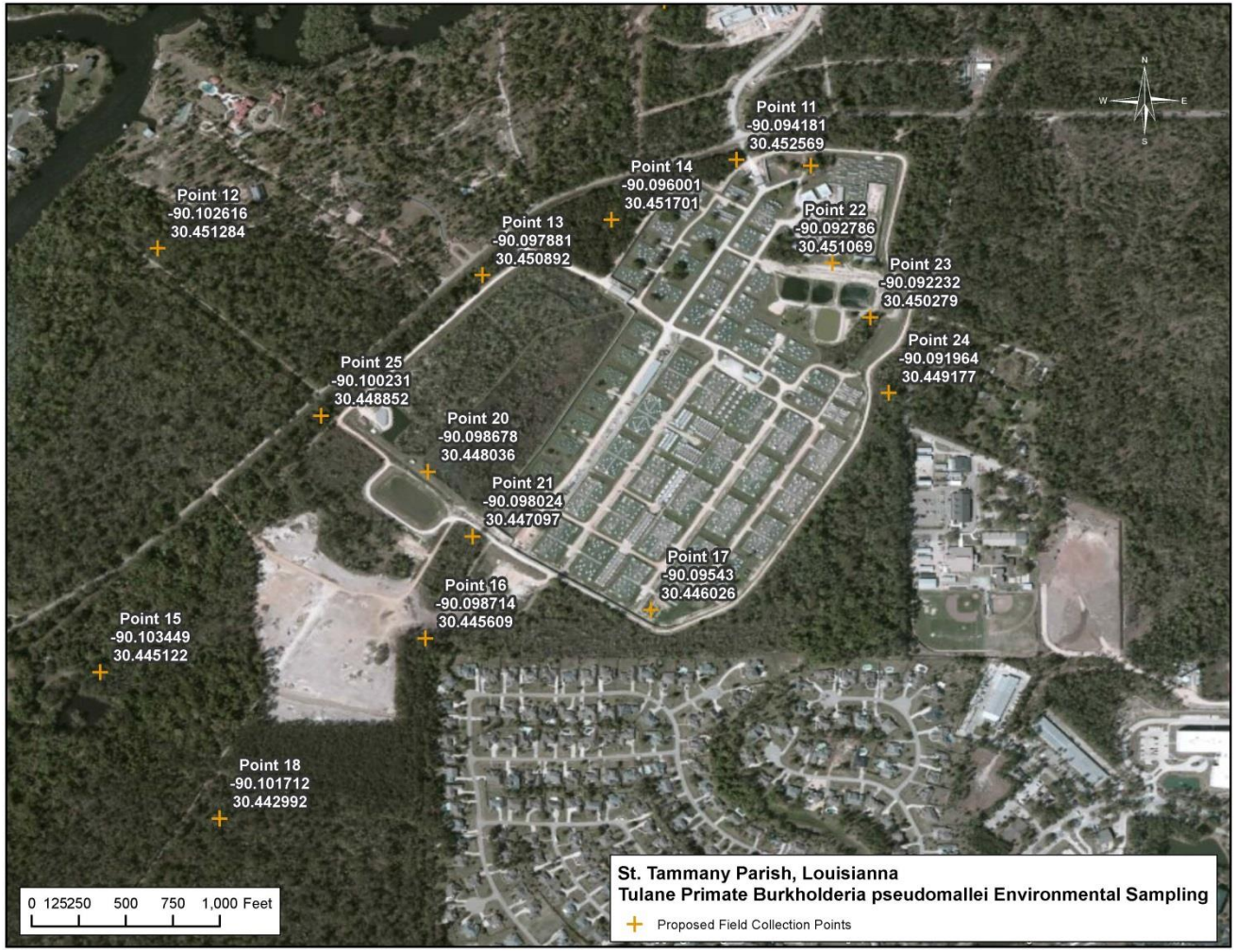


Figure 2 suggested locations for trap placement on the South Campus and outside TNPRC perimeter fence